Supplemental Figure S1-S8:

S1: Western blot analysis of two different polyclonal asymmetric dimethyl arginine methylation (ADMA) antibodies F8216 and BL8241

S2: Western blot analysis of arginine methylation antibodies with different blocking peptides

S3: Beta-actin signal and Coomassie staining of untreated (-) and AdOx treated (+) HCT116 lysates used in western blot analysis of methylation antibodies.

S4: Representative MS/MS spectra of methyl peptides

S5. Motif analysis of ADMA sites identified by two different ADMA antibodies

S6: Venn diagram of overlapping sites identified by different arginine methylation antibodies

S7: Dimethyl lysine antibody specifically enriches dimethyl lysine peptides

S8: Assessment of variance between technical replicates

Supplemental Figure 1.

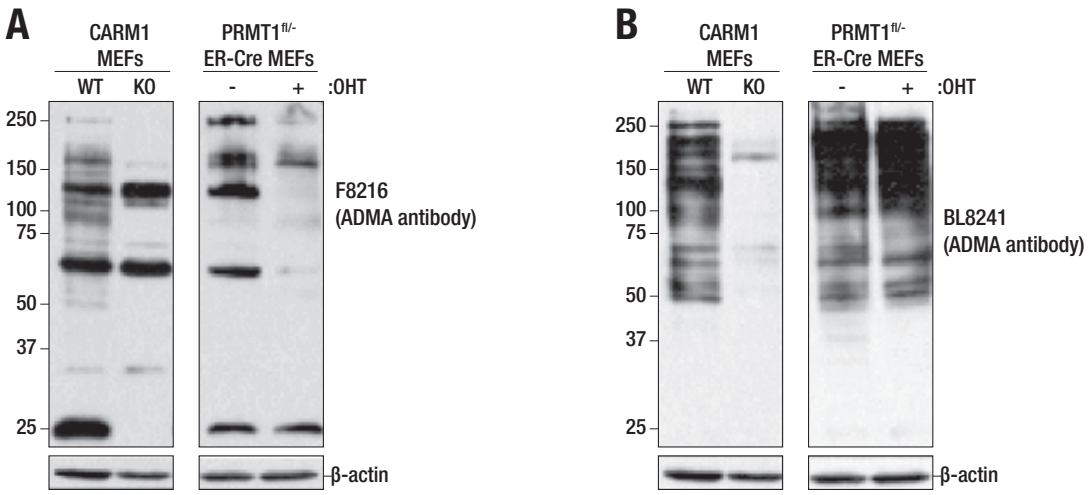


Fig. S1. Western blot analysis of two different polyclonal asymmetric dimethyl arginine methylation (ADMA) antibodies F8216 (A) and BL8241 (B) using lysates from CARM1 wt and knockout (KO) MEF cells, and lysates from PRMT1^{FL}/-ER-Cre MEFs with (+) and without (-) OHT (tamoxifen) treatment. F8216 is more specific to PRMT1 substrates, showing decreased signal in PRMT1 KO cells (PRMT1^{FL}/-ER-Cre, + OHT). BL8241 is more specific to CARM1 substrates, showing decreased signal in CARM1 knockout MEF cells. β-actin signal was used as loading control.

Supplemental Figure 2.

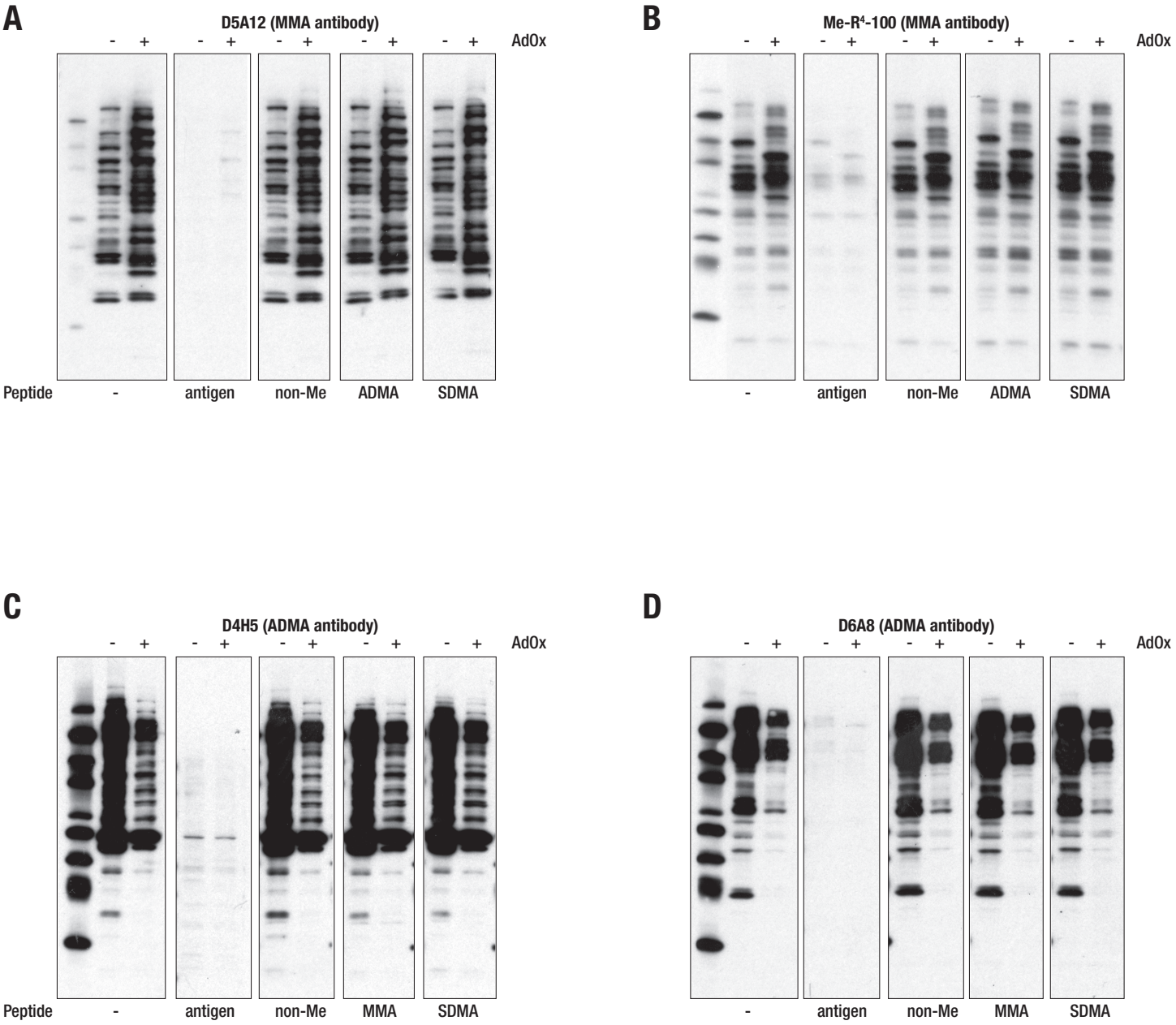


Fig. S2. Western blot analysis of arginine methylation antibodies with different blocking peptides. 30 μg untreated (-) or AdOx (20 μM, 48 hrs) treated (+) HCT116 lysates were used in all the blots. (A, B) Monomethyl arginine antibodies D5A12 and Me-R^4-100 blocked by antigen, non-methyl, ADMA and SDMA peptides. (C, D) Asymmetric dimethyl antibodies D4H5 and D6A8 blocked by antigen, non-methyl, MMA, and SDMA peptides.

Supplemental Figure 3.

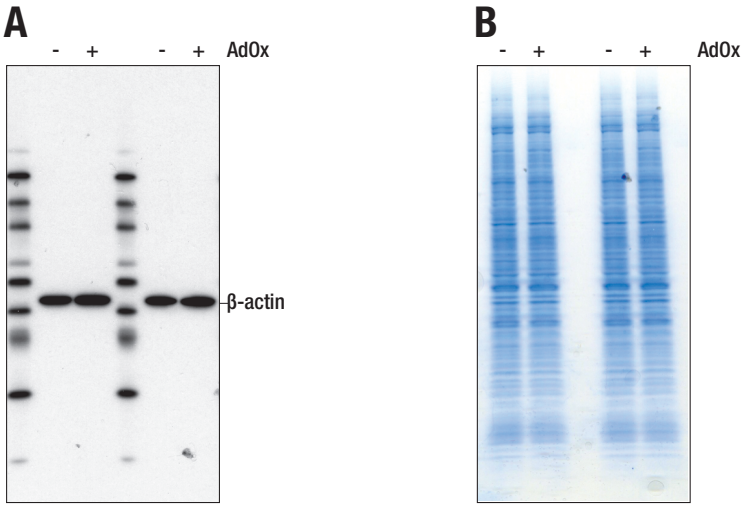


Fig. S3. β-actin signal (A) and Coomassie staining (B) of untreated (-) and AdOx treated (+) HCT116 lysates used in western blot analysis of methylation antibodies. (A) β-actin signal from two representative gels. (B) Coomassie staining of two representative gels.

Supplemental Figure 4.

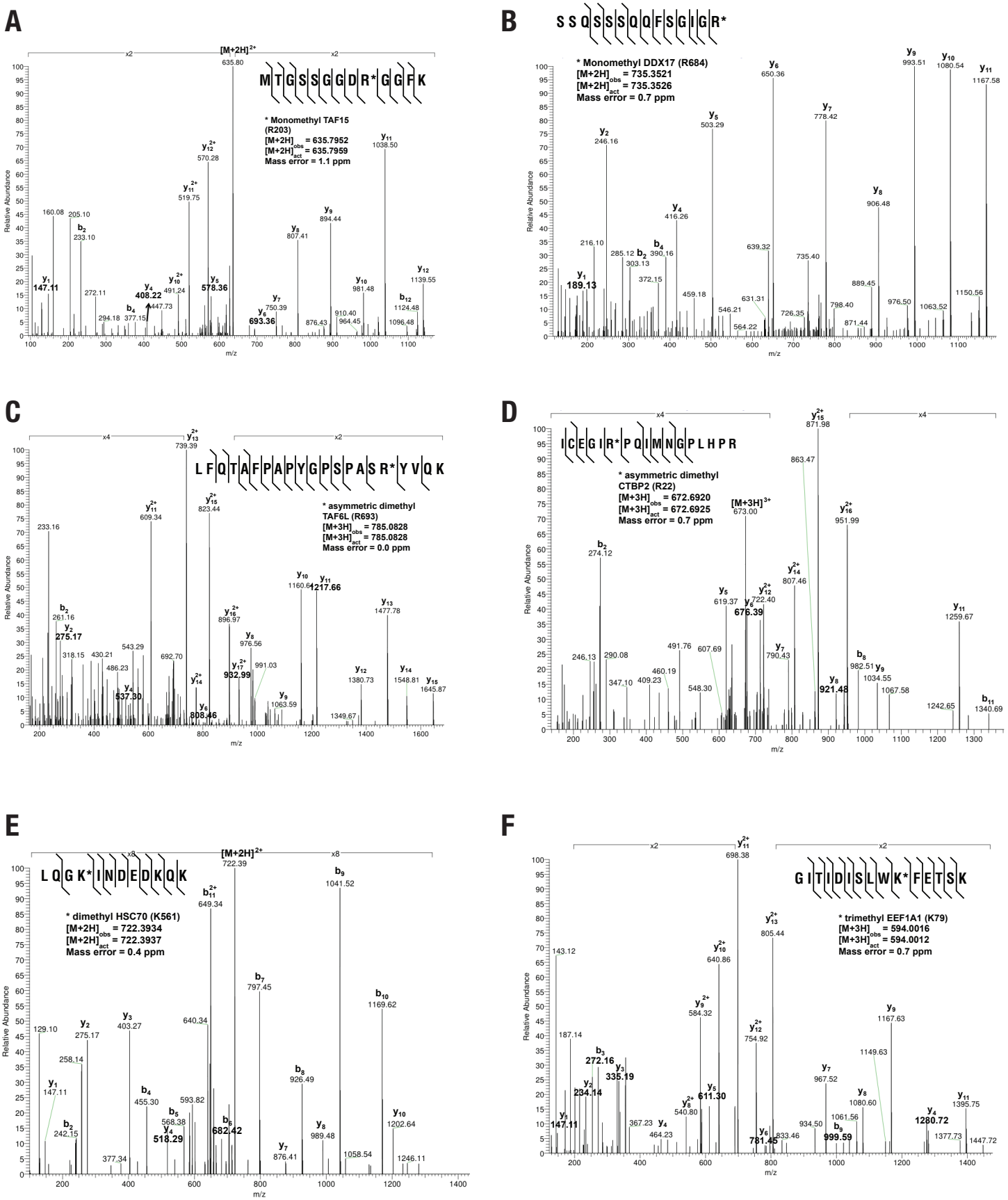


Fig. S4. MS/MS spectra of representative methyl peptides identified in HCT116 cells.

Supplemental Figure 5.

A

B

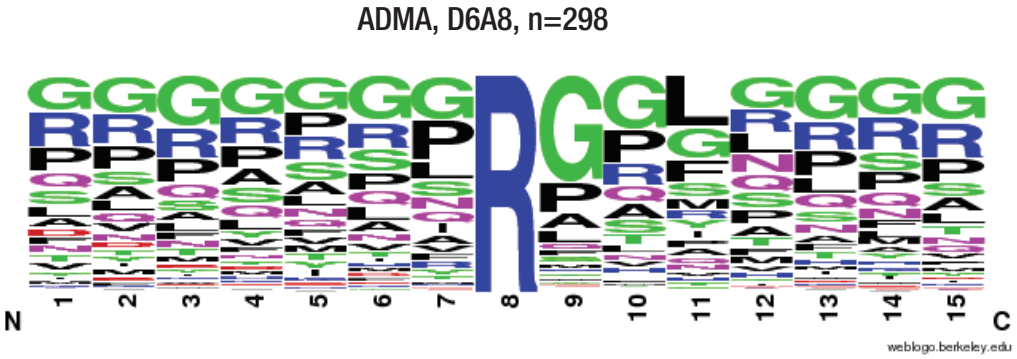


Fig. S5. Motif analysis of arginine methylation sites identified in HCT116 cells by ADMA antibodies D4H5 (A) and D6A8 (B) respectively, using Motif X program ((http://motif-x.med.harvard.edu/), motif logo is drawn with Weblogo (http://weblogo.berkeley.edu/logo.cgi).

Supplemental Figure 6.

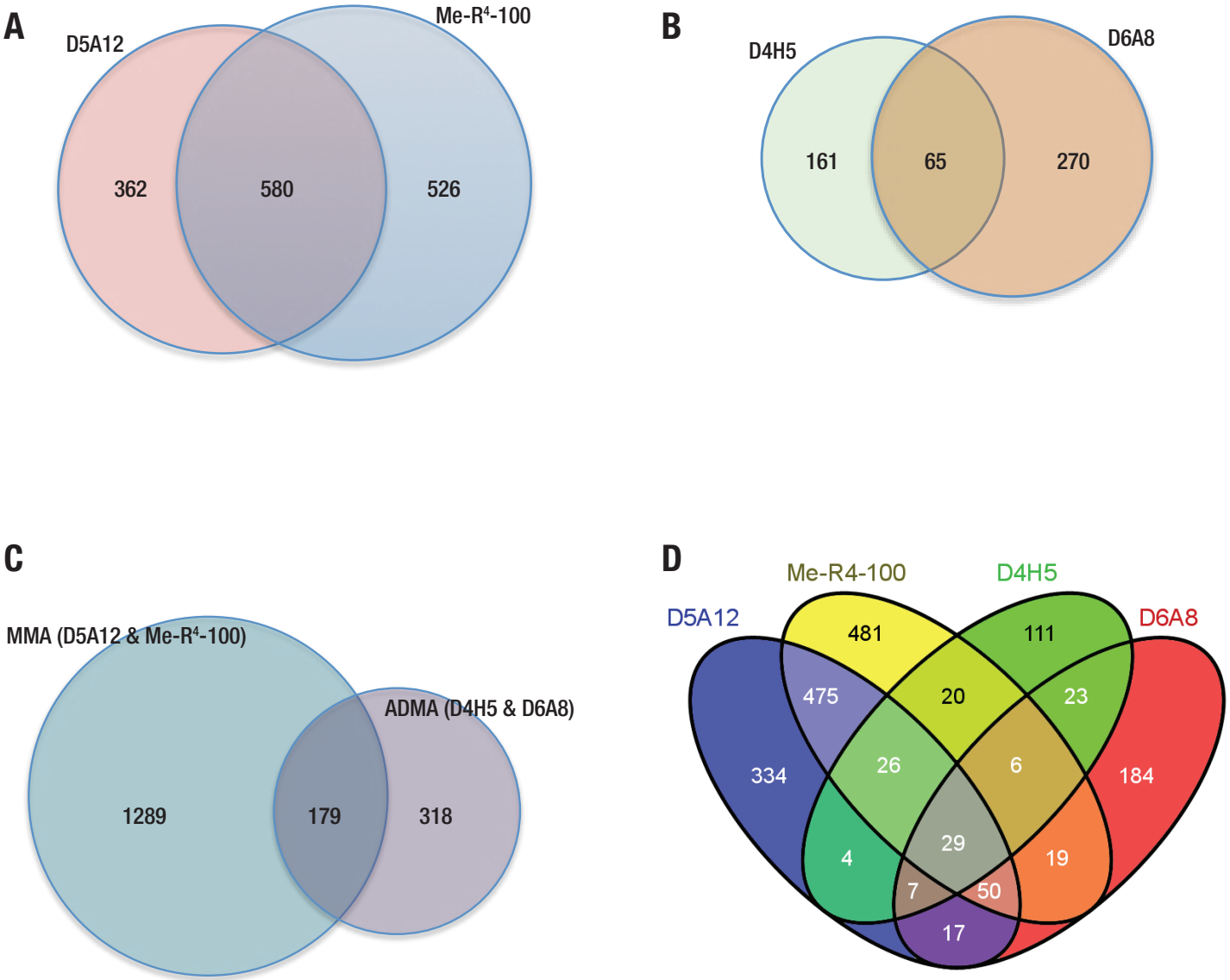
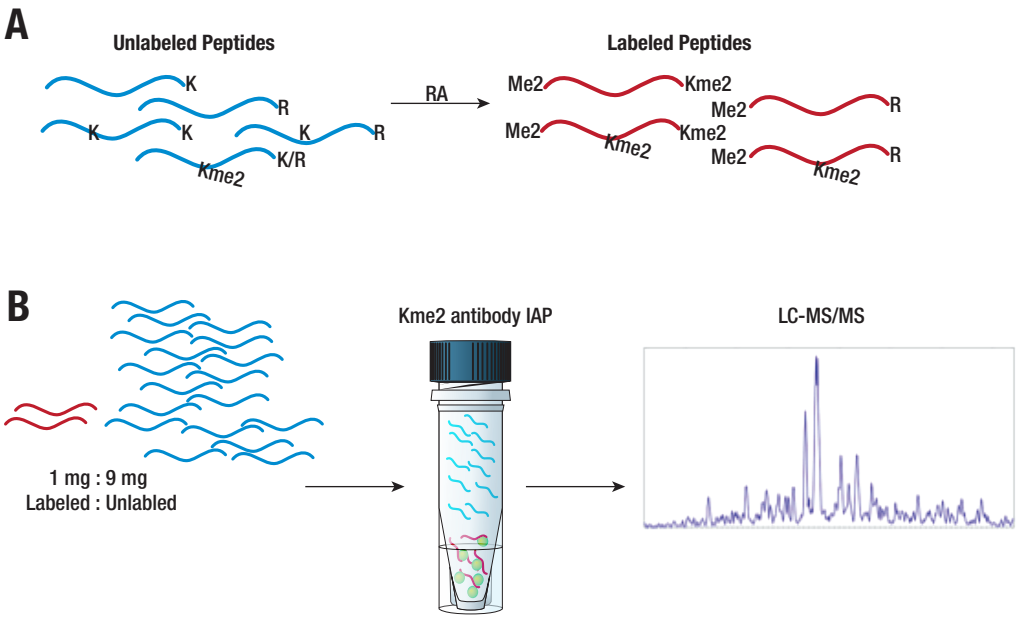


Fig. S6. Venn diagram of overlap between arginine methylation sites identified in HCT116 by different antibodies. (A) Overlap between sites identified by two MMA antibodies Me-R^4-100 and D5A12. (B) Overlap between sites identified by two ADMA antibodies D4H5 and D6A8. (C) Overlap between combined MMA sites and combined ADMA sites. (D) Overlap among sites identified by all 4 arginine methylation antibodies. The 4 set Venn diagram was generated using online program VENNY *(Oliveros, J.C. (2007) VENNY, An interactive tool for comparing lists with Venn Diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html.*

Supplemental Figure 7.



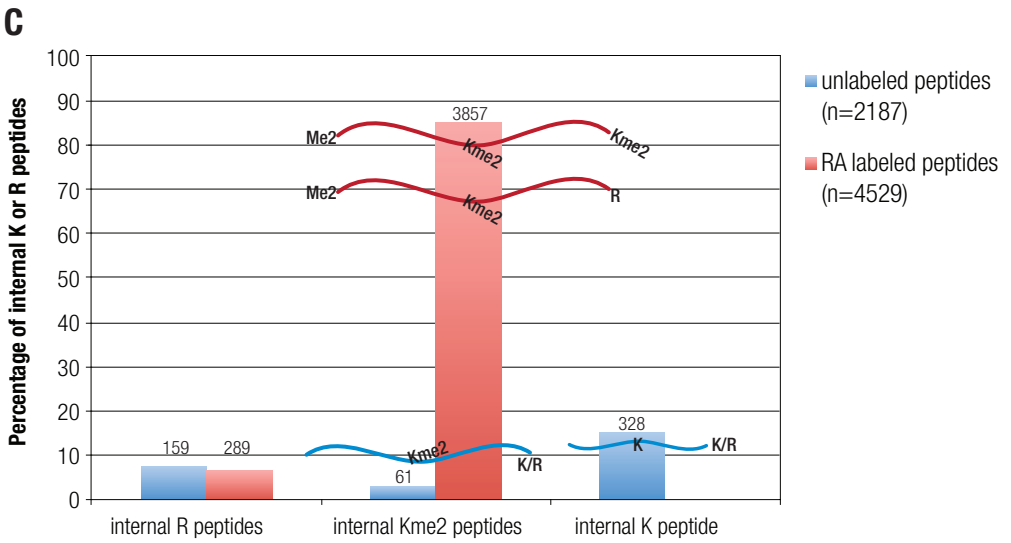


Fig. S7. Dimethyl lysine antibody specifically enriches dimethyl lysine peptides. (A) Reductive amination (RA) reaction labels peptide N-termini and lysine with dimethyl. (B) 1 mg reductive dimethylated peptides from RA reaction and 9 mg of unlabeled HCT116 peptides were used in IAP-LC-MS/MS experiment with dimethyl lysine antibody BL10745. The antibody-enriched peptides were analyzed by LC-MS/MS. (C) 6446 peptides were identified from MS analysis, most of which are RA labeled peptides (N-terminal dimethylated peptides*), although these were 1/10th in the input. Among all N-terminal labeled peptides, 3857 (85%) have internal Kme2. Among unlabeled peptides, 15% (328) have internal lysine, which is the percentage of mis-cleaved lysine residues by trypsin. The percentage of internal arginine peptides are similar in unlabeled peptides and N-terminal labeled peptides, these peptides are mis-cleaved arginine residue peptides resulting from incomplete tryptic digestion. These results show that the dimethyl lysine antibody specifically enriches dimethyl lysine containing peptides. 61 natively occurring di-methyl lysine peptides were also identified in the experiment.

*When a peptide gets N-terminal labeled by RA reaction, all the lysine residues are also dimethylated.

Supplemental Figure 8.

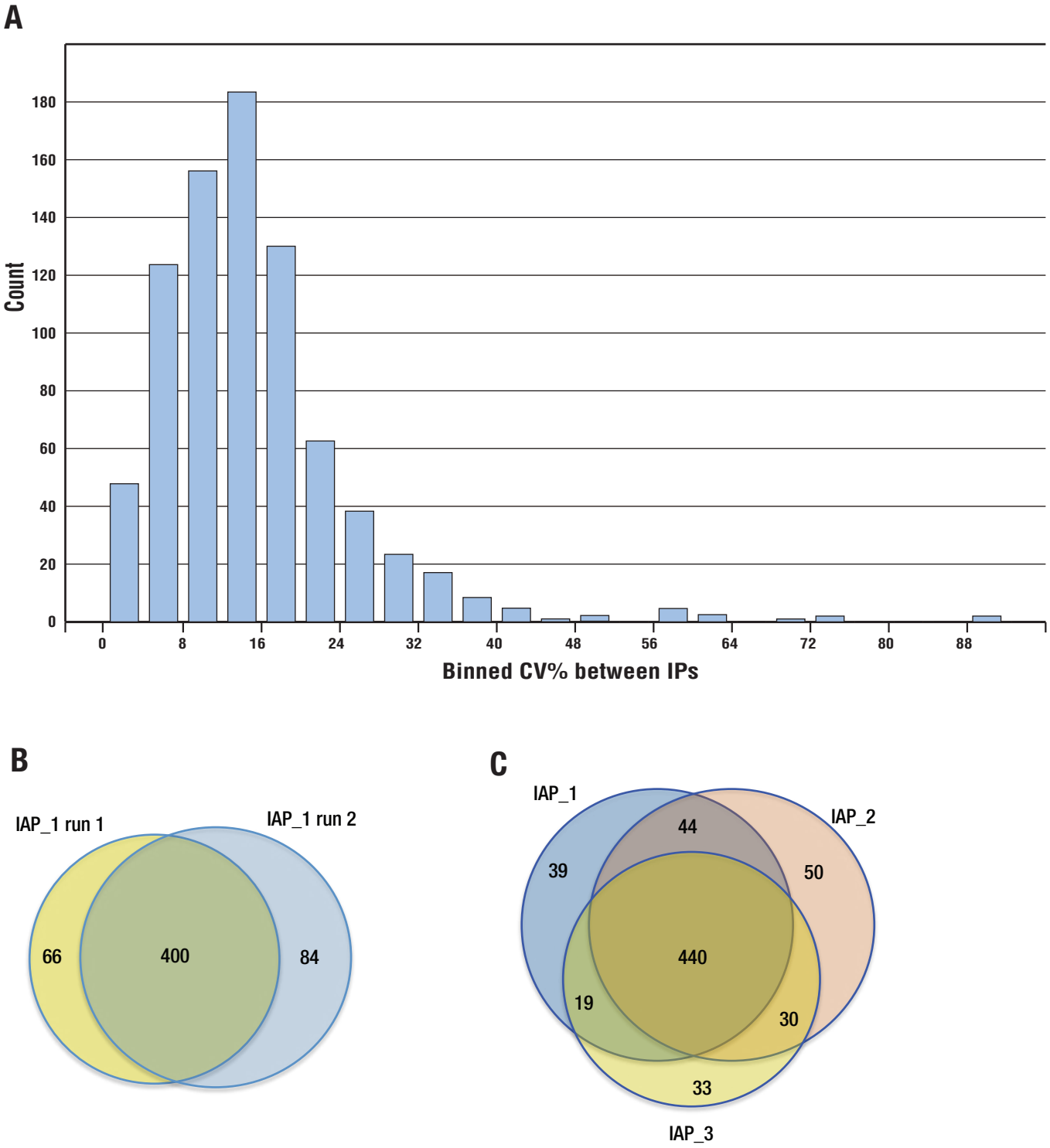


Fig. S8. Assessment of variance between technical replicates. (A) Peptide intensity CV distribution among technical triplicate IAP-MS runs of MMA antibody-enriched methyl peptides. The median %CV across three IAPs was 13%, and more than 80% of the site quantified had %CV lower than 20%. (B) Methylation sites identified from duplicate injections of the same antibody-enriched samples. About 72% of the sites were identified in common. (C) Overlap of methylation sites identified from three IAPs of technical triplicates using the same batch of peptides and same batch of antibodies processed in parallel and run subsequently. About 66% of the sites were identified in common. Sites from one IAP are the sum from two injections of the IAP.